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Salmonellosis in Kentucky Cattle - 2003-2014

Azia Elizabeth Ramirez
University of Kentucky

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Azia Elizabeth Ramirez, Student

David Mannino, MD, Committee Chair

Linda Alexander, EdD, Director of Graduate Studies

Salmonellosis in Kentucky Cattle – 2003-2014

Capstone

A capstone submitted in fulfillment of the
requirements for the degree of Master of Public Health in the
College of Public Health, Department of Environmental Health
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By

Azia Elizabeth Ramirez

Ewing, Virginia

Lab Director: Dr. Craig N. Carter, Veterinary Diagnostic Laboratory, Veterinary Epidemiologist

Lexington, Kentucky

David M. Mannino, MD (Chair)

Wayne T. Sanderson, PhD

Craig N. Carter, DVM

Steven R. Browning, PhD

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LIST OF ABBREVIATIONS

CDC – Centers for Disease Control and Prevention

CFSPH – Center for Food Security and Public Health

CT – Cycle Threshold

eFORS – Electronic Foodborne Outbreak Reporting System

GFN – Global Foodborne Infections Network

IICAB – Institute for International Cooperation in Animal Biologics

IRQ – Interquartile Range

MDR – Multi-Drug Resistance

NORS – National Outbreak Reporting System

PCR – Polymerase Chain Reaction

PMO – Pasteurized Milk Ordinance

S. – Salmonella

SCC – Somatic Cell Count

SPC – Standard Plate Count

UK-VDL – University of Kentucky Veterinary Diagnostic Lab

USDA – United States Department of Agriculture

WHO – World Health Organization

This work is dedicated to Evelyn Ruth Lashuay, to which I owe undying gratitude. I wouldn't be here without you, grandma. I hope you love the flowers. We all miss you.

ABSTRACT

Salmonellosis in Kentucky Cattle – 2003-2014

The group of bacteria known collectively as Salmonella has historically been a major cause of both health and economic hardships. In particular, Salmonella in bovine has been estimated to cause the economy to lose billions of dollars annually, both due to the increased morbidity and mortality rates of the cattle themselves and the indirect loss of potential profits, and also results in the spread of the bacteria to humans to ill effect. Salmonella thus can be quite taxing to agricultural communities, the animals upon which they rely, and the consumers that buy said communities' commodities. Thus, the importance of an understanding of the prevalence and spread of bovine Salmonella in the state of Kentucky, a largely agricultural state that also is a major U.S. beef producer, cannot be understated.

In this study, we present an overview of the status of Salmonella in Kentucky bovine from 2003 to mid-2014. The University of Kentucky Veterinary Diagnostic Lab gathered the data via the voluntary collection of samples that are then analyzed using a variety of tests (PCR, serotyping, isolation, etc) performed in-house. We use descriptive statistics to examine the prevalence of these bacteria in bovine, along with the relative abundances of specific categories of the bacteria within the studies cattle population. This study hopes to gain insight into Salmonella in general, as well as aiding into the development and evolution of public health programs.

CHAPTER 1

INTRODUCTION

Salmonella and Humans

The genus of *Salmonella* is a group of bacteria that have long had substantial effect on humans, domesticated animals, and food. These bacteria are responsible for millions of deaths world wide every year, as well as billions of probable infections, and are easily spread intra-and-inter-species. With incidence rates increasing, as well as the evolution of antibiotic-resistant strains, *Salmonella* poses a substantial risk to the public health and livelihood. (Pui et al. 2011)

This family of bacteria is characterized as facultative anaerobes that are gram-negative and having a rod-like shape; typically, they are 2-3 microns long and 0.4-0.6 microns wide (Pui et al. 2011). Isolated strains have historically been classified based upon the location where it was first isolated (Pui et al. 2011), such as *Dublin*, *Kentucky*, and *Newport* (McGuirk and Peek 2003). A more modern classification scheme is that which is used by the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO), which divide the genus into two separate species, *S. enterica* and *S. bongori*, each with their own group of subspecies. Serovars belonging to *S. enterica* are commonly referred to by a name, while those of *S. bongori* are classified based on antigens in their cell walls and flagella. There are over 2,500 serovars known, however, there is currently no completely standardized nomenclature in the etiology of this bacterial family (CFSPH & IICAB 2005). The bacteria are very resilient to harsh environments, being able to survive and grow at temperatures down to 2-4 ^{o}C and as high as 54 ^{o}C. While they require high water activity for growth, they can survive in very low-moisture environments, such as on dried fruits (Pui et al. 2011). *Salmonella* also has the ability to live significant quantities of time outside of a living host, with some strains surviving almost a

year in the environment and have been successfully isolated in samples of swine meat up to 450 days after slaughter (CFSPH & IICAB 2005).

Salmonella infections, also known as salmonellosis, come in a variety of forms, each with different sets of symptoms and severities. The most common, gastroenteritis, is associated with at least 150 serotypes (Pui et al. 2011) and is characterized by nausea, vomiting, abdominal cramping, and diarrhea (CFSPH & IICAB 2005). Enteric fevers (such as Typhoid) include fever, anorexia, lethargy, and constipation. These are more severe than gastroenteritis, and may result in death (CFSPH & IICAB 2005), however, enteric are typically associated most often with humans (Pui et al. 2011). In approximately 8% of untreated cases, even more extreme infections may occur, with the bacteria passing into the blood stream via the intestinal wall (Pui et al. 2011). The incubation period of salmonellosis depends upon the nature of the infection, with gastroenteritis having a typical incubation period of 12 hours – 3 days, while patients start displaying clinical symptoms of enteric fever 10-14 days after exposure (CFSPH & IICAB 2005).

Salmonella is primarily transferred through direct or indirect contact with fecal matter, but can also be transferred via insects and the eggs of birds, as well as via milk and mammalian utero. Carriers continuously or intermittently shed the bacteria in their feces; Salmonella is also carried asymptomatically in the gastric tract of many species. (CFSPH & IICAB 2005) Poultry, eggs, and dairy product, as well as fruits and vegetables, are major vehicles of transmission for human infection (Pui et al. 2011).

The prevalence and control of harmful pathogens in the workplace, and in particular, agricultural operations, has long been a major sector of public health practice. In a 2013 communications published in the Journal of Dairy Science, the prevalence of animal-borne

pathogens was determined in a sample of dairy farms, testing the bedding, fecal matter, manure storage system, milk filters, soil, and water for a number of microbes. Salmonella was detected at low levels in bedding, fecal matter, and manure storage systems, but was not detected in the milk filters, soil, or water. (Toth et al. 2013) However, in a 2009 review Oliver et al. present the results of similar testing presented in the literature from 2000 to 2008, showing that Salmonella was common in milk filtration systems (Oliver et al. 2009). Kunze et al. 2008, in a study of bovine in the Southern plains of the US, showed that Salmonella was very common on the hide and in the feces of both dairy and beef cattle (Kunze et al. 2008). While there may be disagreement in these three sources, most likely due to the small, inhomogeneous sample of Toth et al., one can see that there is a potential risk to agricultural workers in the dairy and beef industries of contracting salmonellosis and other similar sicknesses.

Direct animal contact is another known transmission route, with Hoelzer, Switt, and Wiedmann describing outbreaks due to human contact with farm animals in the early 2000's (Hoelzer, Switt, and Wiedmann 2011). Cummings et al. wanted to quantify the risk associated with farm animal contact, and in particular, with bovine. Using data collected between 2008 and 2010 in New York and Washington states, they found that contact with farm bovine was highly correlated ($p < 0.001$) with the onset of salmonellosis in both the control and case groups. These two groups were defined based upon if the infecting serovar was or was not commonly associated with cattle. (Cummings et al. 2012) Contact is not isolated to farms, however; another common establishment where Salmonella may spread is a petting zoo. In examining a zoo in Tennessee, it was estimated that 87% of visitors contacted potentially contaminated surfaces, 74% had direct animal contact of any sort, and 49% had hand-to-face

contact. Only 38% of zoo-goers were observed to use hand sanitizer, and 22% were consuming food and beverages in the petting zoo. (Hoelzer, Switt, and Wiedmann 2011)

Salmonella and Cattle

Beyond the immediate public health concerns Salmonella poses to human populations, it also poses a significant economic threat. The USDA's Economic Research Service estimates that, as of 2011, Salmonella is the direct or indirect cause of 2.5 billion dollars in costs to the United States economy; this exceeds both E. coli and Listeria combined. Approximately 40% of all agricultural output on Earth is associated with raising livestock, and approximately 20% of the world's population (1.3 billion people) was employed in this sector as of 2006. In the U.S., there are currently greater than 75,000 operators just in Dairy, and more than 5,000 food animal vets employed by the industry (Cummings et al. 2012). The U.S. and Europe raise the majority of the world's cattle, and the United States is the world's largest producer of beef. (Hoelzer, Switt, and Wiedmann 2011)

The majority of economic losses associated with Salmonella in cattle operations is due primarily to the increased mortality, performance losses, and the cost of medical care. Clinical manifestation of salmonellosis in bovine is typically associated with profuse watery or bloody diarrhea, fever, depression, anorexia, dehydration, and endotoxemia. Less commonly, infected cattle may abort or develop respiratory disease. (Hoelzer, Switt, and Wiedmann 2011) The clinical signs of Salmonellosis typically last 2-7 days (CFSPH & IICAB 2005), however sub-clinical diseases can last in farm bovine populations for months or years, with the animals constantly shedding the disease in their feces. The median shedding duration for Salmonella in dairy cows is 50 days, with an upper limit of 391 days. (Hoelzer, Switt, and Wiedmann 2011)

As noted in the literature, Salmonella can remain in a farm bovine population much longer than the maximum days of fecal shedding, the infections believed to be often endemic to herds, with studies noting high isolation rates in farms. This suggests that cattle herds act as reservoirs to the bacteria. (Hoelzer, Switt, and Wiedmann 2011) It is estimated that 6% of all feedlot cattle harbor Salmonella (CFSPH & IICAB 2005), and according to the 1996 USDA National Health Monitory System Dairy Report, almost 15% of dairy cows were shedding at markets and 66% of all livestock markets had at least one shedding cow (Cummings et al. 2010). While endemicCummings et al. argue that the prevalence of fecal shedding is much higher in herds with clinically symptomatic members (Cummings et al. 2010).

In a 2008 study in Applied and Environmental Microbiology, Kunze et al. studied the prevalence of Salmonella among cattle in the Southern United States. Taking a total of 1,681 samples, with 1081 samples taken from the hide of the abattoir animals, and 600 taken from newly excreted feces from feedlots, they found Salmonella in 55.6% of all the samples. 69.6% of hide samples and 30.3% of fecal samples. There was a total of 22 serovars identified in Kunze et al. 2008, with the most common ones being Anatum, Montevideo, and Cerro. Interestingly, there does seem to be a difference in the antimicrobial resistances (see below) between those animals that had favorable characteristics as compared to those that had less-favorable characteristics (such as the bovine's weight), with none of the samples of the former having resistance to four or more antimicrobial drugs in the former, and 6.5% in the latter.

As mentioned in Section 1 above, Salmonella can be transferred to humans not only through the consumption of beef or direct contact with bovine, but dairy products derived from cow's milk also can be a source of infection. In a 2009 review of pathogens in milk, cow and

otherwise, Oliver et al. show that the increased consumption of raw milk and milk-derived products (i.e. those that do not undergo the pasteurization process) is a public health risk. The authors present the results of all major publications on the prevalence of foodborne pathogens in both bulk raw milk, colostrum, and on inline milk filters between 2000 and 2008. Up to 6.1% of raw milk, 15.0% of colostrum, and 66.0% of inline milk filters tested positive for some serovar of Salmonella, though it is notable that there is a wide spread in the results, with one paper reporting to claim a prevalence rates on filters as low as 1.5%, but with one-sixth of such positive cases being serotyped as Salmonella Typhimurium DT104 (see below). (Oliver et al. 2009)

Oliver et al. 2009 described major outbreaks of foodborne illnesses associated with the consumption of raw milk products between 2000-2008 that have been presented in the Epidemiological literature. There were a total of 202 reported cases in these outbreaks, with 46 hospitalizations. The majority of the hospitalizations occurred as a result of a 2006 and 2007 outbreak in Illinois, spread via the sale of raw milk-derived Cotija cheese sold at a farm supply store, while the 2001 case of multi-drug-resistant Salmonella Newport was due to the consumption of milk that was improperly pasteurized in Connecticut. The examples, along with the generally higher chance of infection from other foodborne pathogens via the consumption of unpasteurized milk-products, suggest public health measures and lobbying for the regulation of bacterial content in such products being sold to American consumers. (Oliver et al. 2009)

The isolation of drug-resistant strains of *Salmonella* first started in the 1960s, with the first examples being resistant to only one antibiotic, while the discovery of MDR (multi-drug resistance) has been observed with increasing frequency recently. (Pui et al. 2011) For example, Cummings et al. 2010 isolated serovars with resistances up to 9 separate drugs. One prime

example of the effect of these new MDR bacteria is S. Typhimurium DT104 , a strain commonly associated with beef contamination whose hospitalization rates are twice that of any other food-borne *Salmonella* (Pui et al. 2011). These antimicrobial-resistant serovars pose such a public health risk, especially in very young (< 5 years of age) and elderly populations, which are more susceptible to severe invasive (potentially fatal) infections, which antibiotics and other such drugs are used to treat (Cummings et al. 2013)

In 2013, Cummings et al. published an article in *Foodborn Pathogens and Disease* detailing the antimicrobial resistances found in Salmonella isolates from dairy cattle in the American Northeast between 2004 and 2011. That data, collected via voluntary submission by Cornell University's Animal Health Diagnostic Center, found a wide range of antimicrobial resistance, with 0% of the 2745 samples being resistant to amikacin, ciprofloxacin, and nalidixic acid, while 72% were resistant to sulfadimethoxine. Overall, there appeared to be a decrease in the prevalence of drug-resistance for most antimicrobials, which the authors explain could be due to an increase in the prevalence of Salmanella Cerro in the region, while not observing any significant trend in either Newport or Typhimurium. (Cummings et al. 2013)

There has been a historical precedent to assume that the rise of such MDR serovars are due to the use and over-use, of antimicrobials in beef and dairy cattle. This seems like a reasonable assumption, since one could easily argue that the use of such drugs results in a evolutionary pressure on the Salmonella bacteria, allowing those more resistant to antibiotics to more readily survive and multiply. Despite many studies concurring with this hypothesis, Cummings et al. 2013 point out that the literature is not unanimous in the impact that the use of antimicrobials have in MDR development, suggesting that the emergence of such resistances may have more variables than predicted. In fact, the authors argue that their sizable data set does

not appear to be consistent with the simple hypothesis linking the use of antibiotics to resistances, attesting to the power of Epidemiology in the study of Salmonella, and the need for further research to aid in the development of public health practices. (Cummings et al. 2013)

National and International Surveillance and Controls

Recommendations published by the World Health Organization to control the spread of food-and-animal borne salmonellosis in humans, last updated online August 2013, focus on primary prevention. (WHO 2013) They suggest that controls be implemented at "all stages of the food chain": producer, handler, and consumer. The WHO emphasizes the importance of good personal hygiene of all individuals involved, while also stressing that agricultural managers and workers should insure that the bacteria can not contaminate farm equipment, crops, or environment. Food handlers and consumers should try to separate raw and cooked meats to eliminate the possibility of cross contamination, while also cooking the animal-derived products thoroughly; they also should ensure the water being used or consumed is safe, or at least boiled. Finally, they stress at multiple instances that one should wash their hands after coming in contact with animals, both wild and domestic, even dogs, cats, and turtles. (WHO 2013)

One way in which Salmonella is controlled and monitored on an international scale is via the WHO's Global Foodborne Infections Network (GFN), a global collaboration dedicated to the improved surveillance and guard against such illnesses. In their 2011 article published in *Foodborne Pathogens and Disease*, Hendriksen et al. discuss such surveillance of 15 of the most common serovars performed in 37 member countries, each of which were determined to have adequate serotyping capabilities (Hendriksen et al. 2011) . The epidemiology in the regions surveyed was complex, but there were some general trends. The two most common serotypes of

Salmonella are Enteritidis (43.5%) and Typhimurium (17.1%), with the latter being more prevalent than the former only in North American and Oceanic nations. Interestingly, there was a general decrease in the proportion of S. Enteritidis in developing countries over the study period, while S. Typhimurium also decreased in the developed world; there was also an increase in the proportion of S. Typhi starting in 2003. Some Salmonella serotypes seemed to be concentrated in specific regions: S. Newport was more often isolated in Latin, North American, and European nations, while Virchow was more prevalent in Asia, Europe, and Oceana. (Hendriksen et al. 2011) These findings are consistent with that which is reported in Herikstad, Motarjemi, and Tauxe 2002, which reported upon a survey of 104 countries by the WHO, finding that, indeed, S. Enteritidis, S. Typhimurium, and S. Thyphi account for over 75% of all serotypes isolated (Heriksen, Motarjemi, and Tauxe 2002). As explained in Hendriksen et al. 2011, such large serotyping surveys allow one to not only understand the current geographical distribution of Salmonella serovars, but also the spread of specific serovars into different regions, both allowing for targeted interventions. (Hendriksen et al. 2011)

One way in which Salmonella and other foodborne diseases have been monitored in the United States has been through the CDC's Foodborne Disease Outbreak Surveillance System. In a report published in June 2013, Gould et al. summarized the finding of this program for cases occurring between 1998 and 2008, which were submitted via either a paper form (1998-2001), electronic form (eFORS, electronic Foodborne Outbreak Reporting System; 2001-2008), or the National Outbreak Reporting System (NORS; after 2008); the reporting cutoff date for the report was in April 2011. There was a total of 13,405 foodborne outbreaks that were reported to the CDC, over half had known etiologies, with 58% of those being related to a contaminated food or ingredient, with 26% confirmed as salmonellosis. An outbreak, as defined by the report, is "two

or more cases of a similar illness caused by the ingestion of a common food" (Gould et al. 2013). While poultry and fish were the most commonly reported culprits of foodborne illness of the 17 predefined food pathways (18.9% and 18.6% respectively), beef was directly after them at 11.9%. It is also interesting to note that there was a significant increase in the outbreaks associated with the consumption of leafy vegetables and dairy in the later years of interest, while the outbreaks associated with egg consumption seemed to be reduced. While the study is informative to a public health official, the authors admit to an incompleteness of its data, and since this surveillance program relies so heavily on voluntary submission, its data may not be uniform to the whole of the US. Likewise, the system is likely to miss smaller outbreaks of illnesses: in 2008, for example, only 7% of Salmonella cases were reported as part of a recognized outbreak. (Gould et al. 2013)

The spread of Salmonella via bovine-derived dairy products is controlled in part by the Pasteurized Milk Ordinance (PMO) of 2007, which defines the quality of the milk via the prevalence of somatic cells (SCC) and the standard plate count (SPC), which requires that milk must have a SCC of < 750,000 per mL, with many advocacy groups trying to further lower the maximum SCC. Oliver et al. 2009 explain that, in general, a higher SCC is typically associated with a higher level of antibiotic residues, pathogens, and toxins in the product, and thus pose a higher threat to the health of American consumers. While the interstate trade of raw dairy products is illegal in the US, the consumption of such products has increased in recent years. Raw milk has similar restrictions on it, with 9 of the 18 States discussed in Oliver et al. 2009 requiring a SPC of less than 20,000 cfu/mL, while some states allow up to 50,000 cfu/mL. The coliform content is also limited by some of the states, with the most stringent being less than 10 cfu/mL,

Salmonella Dublin was endemic in many Danish bovine herds of 2011, and as with many Salmonella serovars, it has proven to be costly to the economic yield and health of herds. Thus, much work has been done in understanding how Salmonella Dublin can be controlled in herds (Nielson et al. 2011) In a 2011 study on management techniques of 84 dairy herds, it was determined that successful control of S. Dublin was correlated with avoiding purchasing cattle from herds that have members that tested positive for the pathogen, while not giving different qualities of colostrum to bull and heifer infants. Almost paradoxically, the authors note that there was a negative correlation between Salmonella and the introducing of such practices as having a separate area for calving and separating the bovine by walls rather than the bars of a stall, which was noted to probably be due to the fact that herds that were already well controlled did not have to introduce such practices. (Nielson et al. 2011) Not only is understanding the effectiveness of certain techniques useful in informing public health officials and veterinarians, but it also has monetary incentive. In Nielson et al. 2012, it was estimated that effective management improved profits: on average over a ten year period, herd owners with excellent control measures would lose 8 euros a year per stall, while those with very poor management would loose on average 188 euros a year per stall. These estimates are just averages, and S. Dublin was shown to cause more severe losses within the first year, with 49 euros per stall in the well-managed herds, and up to 326 euros per stall in poorly managed ones. (Nielson et al. 2012)

CHAPTER 2

MATERIALS AND METHODS

One way to study the Salmonella bacteria and its effect on public health is via measuring its prevalence in specific populations and/or geographic regions. This study analyzes Salmonella samples taken from bovine throughout Kentucky. The data was acquired from the University of Kentucky Veterinary Diagnostic Lab (UK-VDL; vdl.uky.edu), a full service animal diagnostic laboratory that helps service and monitors the animal population of the state. This helps provide regulatory testing for animal populations and sales, as well as acting as an early warning system for any potential epidemics. By offering its services, it takes a unique place in the One Health initiative.

Between 2003 and 2014, the UK-VDL logged 1995 entries that include at least 590 separate cases in Kentucky bovine. The lab also collects the zip code and the breed of the bovine, which allows for cross comparisons between geographic regions and cattle types. While the sample size is quite large and ranges over a large period of time, any attempts to draw conclusions based on it must keep in mind its inherent bias, since samples were given to the lab on a voluntary basis and thus may not necessarily be a representative of Kentucky's domestic bovine population.

The presence of Salmonella in the samples was determined through an aerobic culture of the bacteria, through isolation, or through the use of polymerase chain reaction (PCR) technology. Isolation/culture techniques require a swab of potentially infected material, which are then allowed to multiply and then are examined. A Salmonella PCR, on the other hand, required at least 5 g of fecal matter taken from the bovine in question and then tested for the presence of the Salmonella genome after the amplification of the specific Salmonella sequence.

Of the 1995 entries, 340 were tested using a Salmonella PCR, while the rest were tested either via an aerobic culture or via isolation/serotyping (1429 and 226 respectively). Eliminating duplicates of case numbers and test type (thus eliminating duplicates of the same tests on the same cases) 442 (75%) cases were tested using culturing, 214 (36%) were tested using PCR, and finally 213 (36%) were tested using isolation/ serotyping.

Of those 590 unique case numbers, 248 of them corresponded to bovine breeds commonly associated with beef production, while 196 of them corresponded to dairy cows. 82 cases were specified as a "mixed" feedlot, or of a breed that has traditionally been used for both beef and dairy, while 64 cases were unspecified. The two most common breeds that were declared were Angus, a bovine most commonly used for beef, and Holstein, a dairy cow.

To analyze this data sample, it was imported into Microsoft Excel, with each row representing a separate data sentry. Descriptive statistics along with visual examination of graphs allowed the investigator to make the conclusions presented here.

CHAPTER 3

RESULTS

Figure 1 shows the number of these unique cases as a function of the year they were recorded, which appears to have been fairly constant until recently in 2012. The overall mean value of the number of unique cases was found to be 49.2 with a standard deviation of 29.4 and an interquartile range (IRQ) of 18.3. The overall median value of 41.5, along with the appearance of Figure 1, suggests a skewed distribution. For the years prior to 2013, the mean and median are 40.1 and 41.0 respectively, with a much smaller standard deviation of 11.2 and an IRQ of 15.8. While the data for 2014 was incomplete in the sample being studied, only having data taken for the first few months, the median and mean values for the years 2003-2013 do not differ substantially for 2003-2014.

In addition to what is described above, Figure 1 also shows the number of cases that were tested positive and negative as stacked bars. Surprisingly, while the number of positive cases does not appear to have any substantial correlation with time, the number of negatively tested cases remains near 0 between 2003 and 2011, suddenly increasing in 2012 and peaking in 2013. The majority of 2013 and 2014 cases were found to be negative.

Examining the number of unique cases as a function of the month in which they were entered over the entire data set, there does appear to be an increasing trend in the early part of the year, peaking in the month of March and dropping off in the late spring. Including all the months, one finds an average number of cases to be 49.2 and a median value of 46.0, with a standard deviation of 23.6 and an IRQ of 28.3. If one removes March, this decreases the mean to 44.1, the median to 45.0, the standard deviation to 16.6, and the IRQ to 24.0. The total number of unique March cases (105) is a few times the standard deviation from the mean, and thus may

suggest some notable trend in the receiving of samples. While the exact numbers differ between the 2003-2011 and 2012-2014, this preference for a March is still seen in these two temporal bins.

The results of the PCR tests were most reported with either the resulting CT value or simply a declaration if the sample tested positive or negative. The CT value is a quantitative measurement of the concentration of Salmonella, with a lower value representing a higher concentration than a higher value, and a value above 40 is considered a negative result. Of all PCR tests, 174 were reported as positive, while 166 were reported negative, thus being fairly evenly split between positive and negative. Figure 2 shows the CT value distribution of those tests in which the value was reported (N = 86): 42% were found to have a CT value < 20 (high concentration), 48% were found to have a value of 20-30 (moderate concentration), and 10% were found to have a CT value between 30-40 (low concentration). No CT values were reported for negative cases, and thus suggest that there may be a bias in CT value reporting towards lower CT values, especially since there doesn't appear to be an appreciable difference in the raw number of negative to positive cases. Note that all reported CT values were reported in 2012 or later, with only a total number of 3 PCR tests reported in prior years.

A quick analysis of the reported CT values, as binned into the three groups mentioned in the last paragraph proves fairly interesting, with those below 20 having a mean and median CT value of 18.7 and 18.8 respectively, with a standard deviation of 3.6. Similarly for those with a value of between 20-30, one finds 22.9 and 21.9 for the mean and median, and a standard deviation of 2.6. Finally, for 30-40, one finds a mean and median of 32.4 and 32.2, with a standard deviation of 1.5. The overall mean and median for all PCR tests is 22.2 and 20.75 respectively, with a standard deviation of 4.5, consistent with the idea that the PCR CT value

reporting is skewed towards lower CT values.

While a similar analysis of CT values could not be performed with unique case numbers, since a case may have multiple PCR tests from different samples associated with it, each with a different CT value, one can do a simple comparison between the number of cases that tested positive against those that tested negative. There was a small minority of cases (3 out of 214 unique cases, or ~1.4%) that was found positive for Salmonella in one PCR test, while negative in another. These were considered positively tested cases for the sake of this particular analysis. It was found that 33% (70) of unique cases testing positive, while 67% (144) tested negative. Figure 3 shows the fractional number of unique cases broken up into two temporal ranges: 2012-2014 and 2003-2011. Note that, while there is a huge difference between 2003-2011 and 2012-2014 in terms of the fractional numbers of negative and positive cases, the sample sizes are much different, and thus no real inferences can be made with the 2003-2011 data.

Of the 226 serotyping tests, the most common positive results include Salmonella Typhimurium (19.5%), Salmonella Dublin (12.8%), and Newport (12.8%). For the convenience of our analysis, those serovars with only a single detection, along with those only detected 2-4 times, are collapsed into two more general categories. Those only detected once include Salmonella Berta, Joviana, Johannesburg, Litchfield, Ohio, Paratyphi B., and Senftenberg. Of those serovars that were detected 2-4 times, Salmonella Uganda and Mbandaka having the most cases of this subgroup. There was only one case tested with this method that tested negative, while 3 tested positive for multiple Salmonella serotypes. Finally 16 cases did not have a reported serovar, and thus are not included in our analysis.

Splitting the sample into 2003-2011 (N = 160) and 2012-2014 (N = 49) subsamples, we produce Figure 4, which shows the fractional number of cases for each serotype (or category).

Salmonella Typhimurium (19 %), Newport (16%), and Dublin (11%) are the most common serovars reported for 2003-2011. About 15% of the subsample representing serovars only detected 2-4 times and only 2% are serovars that were only detected once. Serovars wih < 5 detections in this time frame representing this subsample (N = 26) between 2003 and 2011. For 2012-2014 (N = 49), Figure 4 shows that the fractional number of Newport cases decreased in 2012-2014 from 2004-2011, representing only approximately 6%. Salmonella Montevideo increased from 4% in 2003-2011 to 12% in 2012-2014, becoming the third most-common serovar, the other two being Dublin (24%) and Typhimorium (29%).

For the data collected through culturing, one finds 1429 total entries with 442 unique cases. In terms of duplicates, there were only 2 cases where the results differed between tests for the same case, and thus, one does not gain any more insight by examining the results of all tests as compared to all cases as we did for our CT values. There also does not appear to be an appreciable difference in the number of cases between 2003-2011 and 2012-2014, with mean and standard deviations of 38.7 and 11.3 for 2003-2011 and 31.7 and 21.2 for 2012-2014. Note that they are consistent within one standard deviation of scatter, and the smaller mean for 2012-2014 is primarily due to having incomplete data for 2014.

Similar to before, the results from the culturing tests were analyzed by removing duplicates. Due to their small number (2 cases), the cases which showed different results through different culturing tests were eliminated from the analysis, bring N = 442 to 440. Over the entire length of the study, the fraction of positive to negative cases, showing that for this type of test, the data favor strongly a positive result (408, 93%) as compared to a negative result (32, 7%) for the entire length of this study. However, splitting this sample into two bins as we have done previously (2003-2011 and 2012-2014), we do find a significant difference between the

fraction of positive and negative cases for these two temporal bins: 99% of all culturing cases were tested positive between 2003-2011, while for 2012-2014, 68% of cases were tested positive.

The culture testing also gave the group of Salmonella for most of the cases, with only approximately 13% of positive cases being labeled with an unspecified group of Salmonella. The most prevalent cases over the entire time of study are from Salmonella Group B and C. Breaking Salmonella Group C into three separate subgroups, one finds that the most prevalent was Salmonella Group C2 (40% of 124 Group C cases), while no single subgroup seems overly overabundant; Salmonella Group C1 corresponds to 36% and Salmonella Group C3 corresponds to 24%. For Salmonella Group E, Group E1 and E2 are equally abundant at 47% each, leaving Salmonella Group E4 only taking up about 6% of all Salmonella Group E cases.

Figure 5 shows these results after separating the culturing cases into the two typical temporal bins (2003-2011 and 2012-2014), illustrating that most of the cases unsurprisingly come from 2003-2011 and contains most of the samples that were tested positive for Group B or Group C Salmonella. For 2012-2014, however, there is a large increase in the fraction of null-tested cases and a significant decrease in the fractional number of Salmonella Group B.

CHAPTER 4

CONCLUSIONS

The previous two sections have presented our study on salmonella in Kentucky bovine between 2003 and mid-2014, with data collected by the University of Kentucky Veterinary Diagnostic Lab. Based upon this analysis, summarized in Figures 1-5, one can see that the data collected on bovine salmonella has been fairly inhomogeneous over the time frame being studied. While prior to 2012 nearly all unique case numbers correspond to being tested positive, the later three years of show a substantial number of negative cases, with the majority of cases in 2013 and 2014 corresponding to a null result.

The large increase in the total number of cases as seen in 2012-2014 most likely corresponds to an increase in the number of bovine being tested. The cause of this increase is not able to be completely ascertained from the data, however it is reasonable to assume it was either due to either an increased familiarity of the services provided by the UK-VDL and/or an increased caution against bovine salmonellosis amongst beef and dairy farmers.

The right side of Figure 1 also may suggest a downward trend in the number of unique positive cases, with the noteable decrease from 2011-2013, with 2014 being of course incomplete. However, at the lowest point with complete data (2013), the number of positive cases is at most one standard deviation from the average of 2003-2011. This, along with a lack of data passed mid-2014, leads to an inadequacy in the data to support this hypothesis, and thus one cannot verify this supposed downward trend with reasonable rigor.

Examining the cases by test type, one finds a number of interesting results. For example, focusing on the PCR testing, one finds that there is a skewed distribution in CT values, suggesting that, of the CT values reported, they are more heavily concentrated towards lower

values, and thus larger number of salmonella in the sample. However, when investigating this further, one finds that not all CT values were reported by the scientists performing the procedure, and that there was no null case where a CT value was reported. Thus, while it is true that one observes a skew in CT values, it is reasonable to assume this to be due to a bias in the sampling of the values, with higher CT values going unreported. In terms of the positive and negative tests, 33% of the cases that were tested with PCR tested positive, while 67% tested positive. Almost all of these cases were from after 2011, with only 3 cases being reported prior.

Examining the serotyping and culturing results allows one to discover the prevalence and trends of different strains of salmonella. For both 2003-2011 and 2012-2014, S. Typhimorium is the most prevalent serotype of salmonella detected, while a large increase in the fractional prevalence is observed in S. Dublin The culturing results show a large decrease in the fractional prevalence of Group B Salmonella and Group C Salmonella, while there was a small increase in Group D Salmonella.

In conclusion, while positive results were not as numerous as one would have hoped in this study, one may still make some important inferences from it. The UK-VDL has provided an important public health service to the agricultural community of Kentucky, and its services appear to be becoming more heavily utilized by the owners of the state's numerous cattle feedlots. As Kentucky is a major beef producer, the screening for salmonella in the state's cattle is important from both a economic and public health standpoint, and thus this increase in this service's utilization is promising. The increase in the prevalence in S. Dublin is not entirely unsurprising, since this is one of the most prevalent salmonella serotypes, however, it is noteworthy that S. Dublin in particular is particularly costly to feedlots (Nielson et al. 2012). As such, focusing on targeting and treating against this particular serotypes might be beneficial to

the health of Kentucky cattle and consumers of Kentucky bovine-derived products.

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CITATIONS

CNFSPH & IICAB 2005, The Center For Food Security and Public Health & Institute For International Cooperation in Animal Biologics, Iowa State University, College of Veterinary Medicine, http://www.cfsph.iastate.edu/Factsheets/pdfs/nontyphoidal_salmonellosis.pdf

Cummings, Kevin J. et al. 2010, Foodborne Pathogens and Disease, 7, 7, p. 815

Cummings, Kevin J. et al. 2012, Emerging Infectious Disease, 18, 12, p. 1929

Cummings, Kevin J. et al. 2013, Foodborne Pathogens and Disease, 10, 4, p. 353

Hendriksen, Rene S. et al. 2011, Foodborne Pathogens and Disease, 8, 8, p. 887

Herikstad, H., Motarjemi, Y., Tauxe, R. V. 2002, Epidemiology & Infection, 129, p. 1

Hoelzer, Karin, Switt, Andrea, & Widmann, Martin 2011, Veterinary Research, 42, 34

Gould et al. 2013, Surveillance for Foodborne Disease Outbreaks – United States, 1998-2008, Morbidity and Mortality Weekly Report, Centers for Disease Control, http://www.cdc.gov/mmwr/preview/mmwrhtml/ss6202a1.htm

Kunze, David J. et al. 2008, Applied and Environmental Microbiology, Jan, p. 345

McGuirk, Sheila M. & Peek, Simon 2003, Preconvention Seminar 7: Dairy Herd Problem Investigation Strategies, American Association of Bovine Practitioners 36th Annual Conference, http://www.vetmed.wisc.edu/dms/fapm/fapmtools/7health/Salmorev.pdf

Nielson, T.D. et al. 2012, Preventative Veterinary Medicine, 105, p. 101

Oliver, Stephen P. et al. 2009, Foodborne Pathogens And Disease, 6, 7, p. 793

Pui, C.F. et al. 2011, International Food Research Journal, 18, p. 465

Toth, J.D. et al. 2013, Journal of Dairy Science, 96, p. 5756

UK-VDL, University of Kentucky Veterinary Diagnostic Laboratory, http://vdl.uky.edu/

WHO 2013, World Health Organization, Salmonella (non-typhoidal),

http://www.who.int/mediacentre/factsheets/fs139/en/

FIGURES

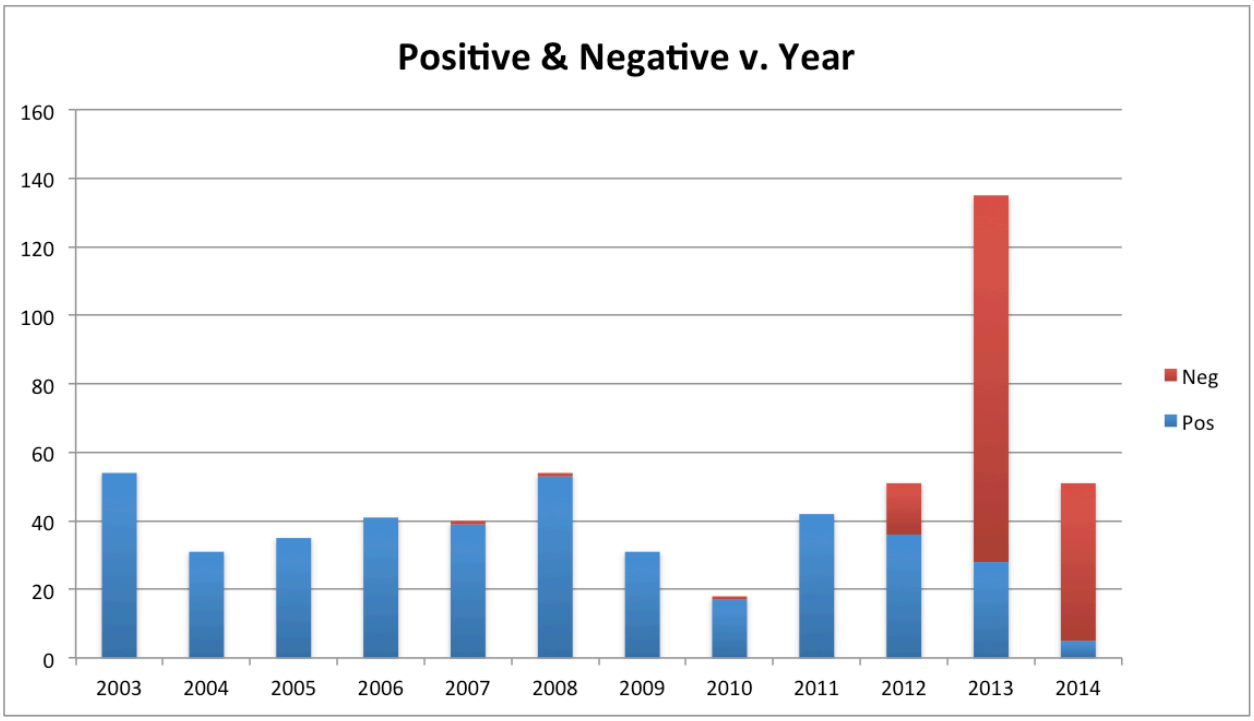

Figure 1

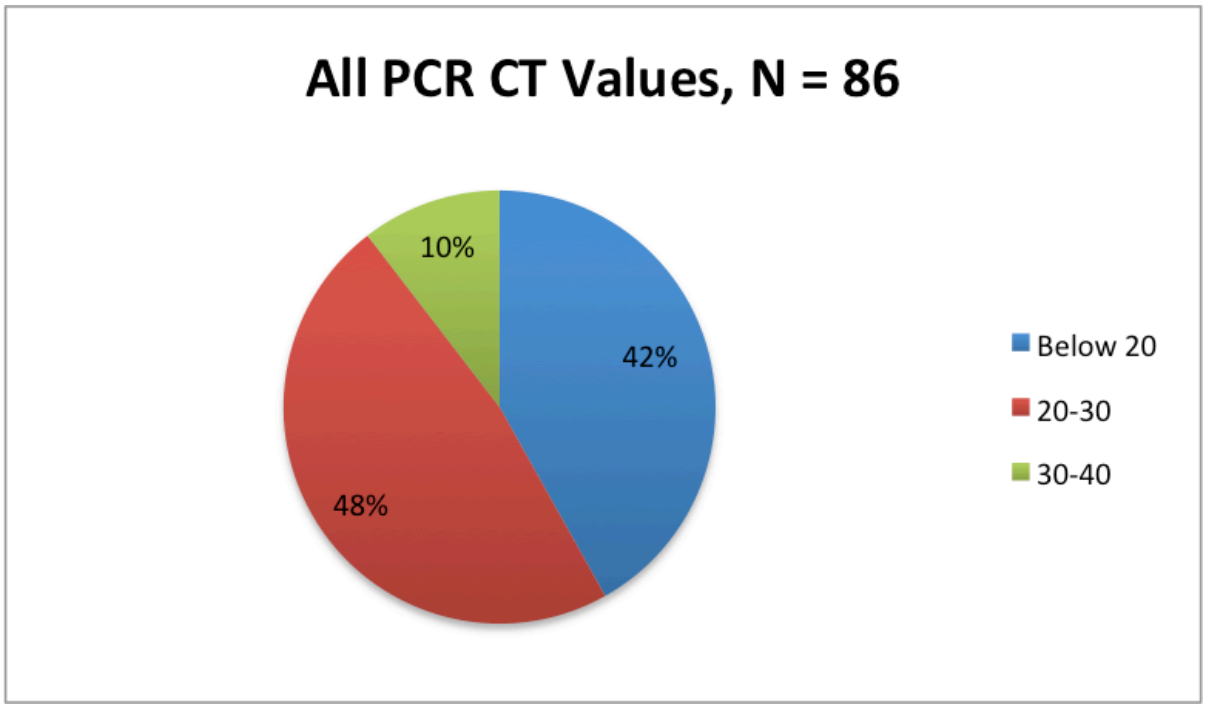

Figure 2

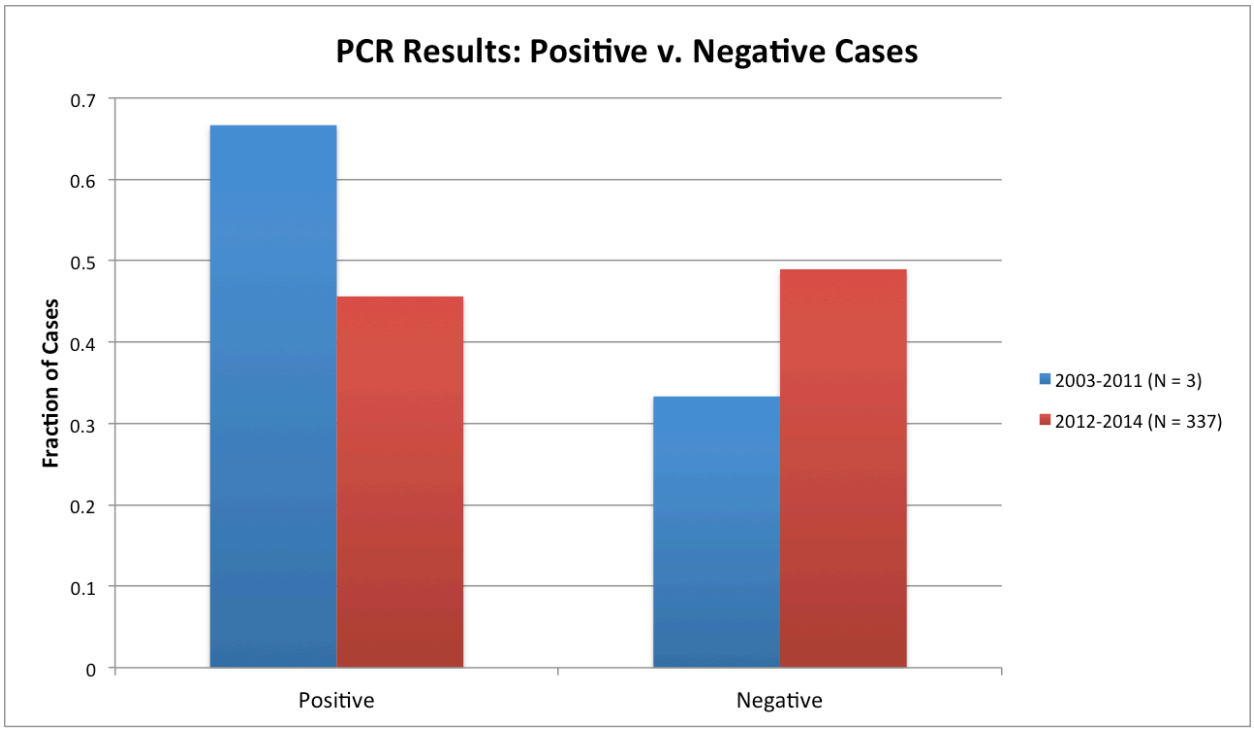

Figure 3

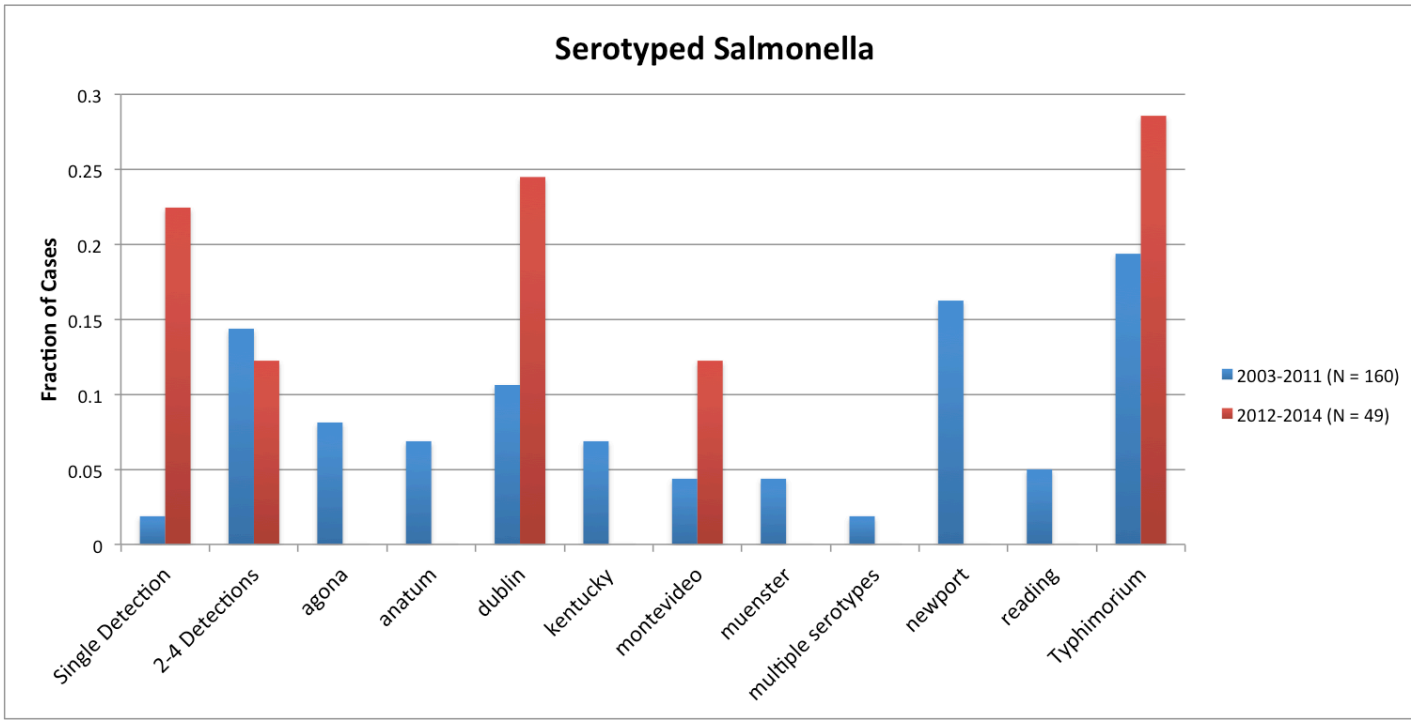

Figure 4

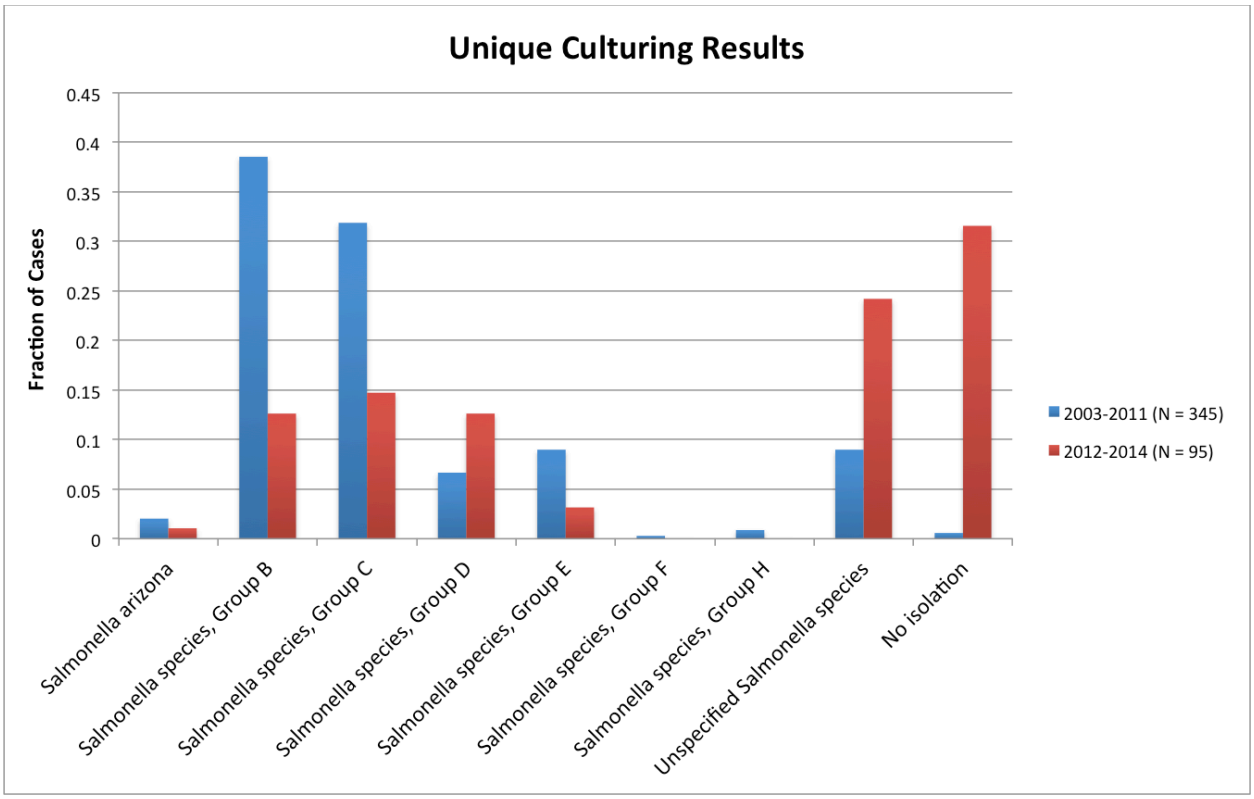
Unique Culturing Results
Fraction of Cases
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Salmonella arizona
Salmonella species, Group B
Salmonella species, Group C
Salmonella species, Group D
Salmonella species, Group E
Salmonella species, Group F
Salmonella species, Group H
Unspecified Salmonella species
No isolation
2003-2011 (N = 345)
2012-2014 (N = 95)

Figure 5

Biographical Sketch

Azia Elizabeth Ramirez was born Azia Elizabeth Routson on October 29, 1987 and was raised by her maternal grandparents, David and Evelyn Lashuay in rural Wayne, OH. As a child and teenager, she developed a love of animals and the rural community around her, participating in numerous agricultural clubs and becoming a competitive rabbit breeder and horse rider. After graduating from Elmwood High School, Azia attended the University of Findlay in Findlay, OH as a Pre-Veterinary and Biology student. While there, however, she suffered a major hemorrhagic stroke that would change her life, resulting in a menagerie of health problems. Despite this, Azia was able persevere with the help of her loving fiancé (now husband) Alejandro Ramirez, graduating in 2012 and continuing her education at the University of Kentucky – College of Public Health immediately after.

Azia is currently attending Lincoln Memorial University as a Veterinary student, and hopes to use her public health and veterinary knowledge to give back to the agricultural community and the creatures upon which we all depend. She is a proud Navy wife and the mother to one dog (Mocha) and three cats (Latte, Cinder, and Minchi).